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DERWENT-ACC-NO: 1998-230234
 DERWENT-WEEK: 200482
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TITLE: Host cell containing recombinant expression vector encoding Clostridium botulinum type B or E toxin - useful to treat humans and other animals at risk of intoxication with clostridial toxin

INVENTOR: THALLEY, B S; WILLIAMS, J A

PATENT-ASSIGNEE: OPHIDIAN PHARM INC (OPHIN), ALLERGAN BOTOX LTD (ALLR),
ALLERGAN INC (ALLR), ALLERGAN SALES INC (ALLR)

PRIORITY-DATA: 1996US-0704159 (August 28, 1996), 1995US-0405496 (March 16, 1995), 2003US-0354774 (January 30, 2003), 2002US-0271012 (October 15, 2002), 2003US-0729122 (December 5, 2003), 2003US-0729039 (December 5, 2003), 2003US-0729527 (December 5, 2003), 2003US-0727898 (December 4, 2003), 2003US-0728696 (December 5, 2003)

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PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
<input type="checkbox"/> <u>US 20040253673 A1</u>	December 16, 2004		000	C07K014/33
<input type="checkbox"/> <u>WO 9808540 A1</u>	March 5, 1998	E	427	A61K039/00
<input type="checkbox"/> <u>AU 9742450 A</u>	March 19, 1998		000	
<input type="checkbox"/> <u>EP 1105153 A1</u>	June 13, 2001	E	000	A61K039/00
<input type="checkbox"/> <u>US 20030215468 A1</u>	November 20, 2003		000	A61K039/08
<input type="checkbox"/> <u>US 20030219457 A1</u>	November 27, 2003		000	C12Q001/68
<input type="checkbox"/> <u>US 20040115215 A1</u>	June 17, 2004		000	A61K039/395
<input type="checkbox"/> <u>US 20040142455 A1</u>	July 22, 2004		000	C12P021/02
<input type="checkbox"/> <u>US 20040219637 A1</u>	November 4, 2004		000	C12P021/02
<input type="checkbox"/> <u>US 20040235118 A1</u>	November 25, 2004		000	C12P021/04

DESIGNATED-STATES: AU CA JP AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE
 AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
US20040253673A1	March 16, 1995	1995US-0405496	CIP of
US20040253673A1	August 28, 1996	1996US-0704159	Cont of
US20040253673A1	October 15, 2002	2002US-0271012	Div ex
US20040253673A1	December 5, 2003	2003US-0728696	
US20040253673A1		US 5919665	CIP of
WO 9808540A1	August 28, 1997	1997WO-US15394	

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AU 9742450A		WO 9808540	Based on
EP 1105153A1	August 28, 1997	1997EP-0940746	
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US20030215468A1	March 16, 1995	1995US-0405496	CIP of
US20030215468A1	August 28, 1996	1996US-0704159	Cont of
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US20030215468A1		US 5919665	CIP of
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US20030219457A1	August 28, 1996	1996US-0704159	Cont of
US20030219457A1	October 15, 2002	2002US-0271012	
US20030219457A1		US 5919665	CIP of
US20040115215A1	March 16, 1995	1995US-0405496	CIP of
US20040115215A1	August 28, 1996	1996US-0704159	Cont of
US20040115215A1	October 15, 2002	2002US-0271012	Div ex
US20040115215A1	December 5, 2003	2003US-0729122	
US20040115215A1		US 5919665	CIP of
US20040142455A1	March 16, 1995	1995US-0405496	CIP of
US20040142455A1	August 28, 1996	1996US-0704159	Cont of
US20040142455A1	October 15, 2002	2002US-0271012	Div ex
US20040142455A1	December 5, 2003	2003US-0729039	
US20040142455A1		US 5919665	CIP of
US20040219637A1	March 16, 1995	1995US-0405496	CIP of
US20040219637A1	August 28, 1996	1996US-0704159	Cont of
US20040219637A1	October 15, 2002	2002US-0271012	Div ex
US20040219637A1	December 5, 2003	2003US-0729527	
US20040219637A1		US 5919665	CIP of
US20040235118A1	March 16, 1995	1995US-0405496	CIP of
US20040235118A1	August 28, 1996	1996US-0704159	Cont of
US20040235118A1	January 30, 2003	2003US-0354774	Div ex
US20040235118A1	December 4, 2003	2003US-0727898	
US20040235118A1		US 5919665	CIP of

INT-CL (IPC): A61 K 38/08; A61 K 39/00; A61 K 39/02; A61 K 39/08; A61 K 39/12; A61 K 39/38; A61 K 39/395; C07 H 21/04; C07 K 14/33; C07 K 16/00; C07 K 16/12; C12 N 1/18; C12 N 1/21; C12 N 5/06; C12 N 15/00; C12 N 15/09; C12 N 15/63; C12 N 15/70; C12 N 15/74; C12 P 21/02; C12 P 21/04; C12 P 21/06; C12 P 21/08; C12 Q 1/68

RELATED-ACC-NO: 1994-217494;1994-271898 ;1994-341765 ;1996-230603

ABSTRACTED-PUB-NO: WO 9808540A
BASIC-ABSTRACT:

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Host cell, containing a recombinant expression vector, which encodes a protein comprising at least a portion of a Clostridium botulinum type B or E toxin, is claimed. Also claimed are: (1) a host cell containing a recombinant expression vector, which encodes a fusion protein comprising a non-toxin protein sequence, preferably comprising a poly-histidine tract, and at least a portion, preferably comprising the receptor binding domain, of a C. botulinum type B or E toxin; and (2) a vaccine, preferably endotoxin free, comprising the fusion protein of (1), and preferably further comprising a fusion protein comprising a non-toxin protein sequence and at least a portion of C. botulinum type A toxin.

USE - An antigen comprising the fusion protein can be used to generate a novel antibody (Ab) directed against a C. botulinum toxin (claimed). The vaccine and the Ab can be used to treat humans and other animals at risk of intoxication with clostridial toxin, while the Ab or the protein can also be used for the detection of bacterial toxins.

ABSTRACTED-PUB-NO: WO 9808540A
EQUIVALENT-ABSTRACTS:

CHOSEN-DRAWING: Dwg.0/40

DERWENT-CLASS: B04 D16

CPI-CODES: B04-E08; B04-G01; B04-N0300E; B12-K04A4; B14-A01; B14-S11B; D05-H07; D05-H11; D05-H14A1; D05-H17C;

or fused with another protein or protein domain, the fusion partner, to allow for enhanced stability of the protein of interest and/or ease of purification of the fusion protein.

As used herein, the term "maltose binding protein" refers to the maltose binding protein of *E. coli*. A portion of the maltose binding protein may be added to a protein of interest to generate a fusion protein; a portion of the maltose binding protein may merely enhance the solubility of the resulting fusion protein when expressed in a bacterial host. On the other hand, a portion of the maltose binding protein may allow affinity purification of the fusion protein on an amylose resin.

As used herein, the term "poly-histidine tract" when used in reference to a fusion protein refers to the presence of two to ten histidine residues at either the amino- or carboxy-terminus of a protein of interest. A poly-histidine tract of six to ten residues is preferred. The poly-histidine tract is also defined functionally as being a number of consecutive histidine residues added to the protein of interest which allows the affinity purification of the resulting fusion protein on a nickel-chelate column.

As used herein, the term "purified" or "to purify" refers to the removal of contaminants from a sample. For example, antitoxins are purified by removal of contaminating non-immunoglobulin proteins; they are also purified by the removal of immunoglobulin that does not bind toxin. The removal of non-immunoglobulin proteins and/or the removal of immunoglobulins that do not bind toxin results in an increase in the percent of toxin-reactive immunoglobulins in the sample. In another example, recombinant toxin polypeptides are expressed in bacterial host cells and the toxin polypeptides are purified by the removal of host cell proteins; the percent of recombinant toxin polypeptides is thereby increased in the sample. Additionally, the recombinant toxin polypeptides are purified by the removal of host cell components such as lipopolysaccharide (e.g., endotoxin).

The term "recombinant DNA molecule" as used herein refers to a DNA molecule which is comprised of segments of DNA joined together by means of molecular biological techniques.

The term "recombinant protein" or "recombinant polypeptide" as used herein refers to a protein molecule which is expressed from a recombinant DNA molecule.

The term "native protein" as used herein refers to a protein which is isolated from a natural source as opposed to the production of a protein by recombinant means.

As used herein the term "portion" when in reference to a protein (as in "a portion of a given protein") refers to fragments of that protein. The fragments may range in size from four amino acid residues to the entire amino acid sequence minus one amino acid.

As used herein "soluble" when in reference to a protein produced by recombinant DNA technology in a host cell is a protein which exists in solution in the cytoplasm of the host cell; if the protein contains a signal sequence the soluble protein is exported to the periplasmic space in bacteria hosts and is secreted into the culture medium in eucaryotic cells capable of secretion or by bacterial host possessing the appropriate genes (i.e., the *kil* gene). In contrast, an insoluble protein is one which exists in denatured form inside cytoplasmic granules (called inclusion bodies) in the host cell. High level expression (i.e., greater than 10–20 mg recombinant protein/liter of bacterial culture) of recombinant proteins often results in the expressed protein being found in inclusion bodies in the bacterial host cells. A

soluble protein is a protein which is not found in an inclusion body inside the host cell or is found both in the cytoplasm and in inclusion bodies and in this case the protein may be present at high or low levels in the cytoplasm.


A distinction is drawn between a soluble protein (i.e., a protein which when expressed in a host cell is produced in a soluble form) and a "solubilized" protein. An insoluble recombinant protein found inside an inclusion body may be solubilized (i.e., rendered into a soluble form) by treating purified inclusion bodies with denaturants such as guanidine hydrochloride, urea or sodium dodecyl sulfate (SDS). These denaturants must then be removed from the solubilized protein preparation to allow the recovered protein to renature (refold). Not all proteins will refold into an active conformation after solubilization in a denaturant and removal of the denaturant. Many proteins precipitate upon removal of the denaturant. SDS may be used to solubilize inclusion bodies and will maintain the proteins in solution at low concentration. However, dialysis will not always remove all of the SDS (SDS can form micelles which do not dialyze out); therefore, SDS-solubilized inclusion body protein is soluble but not refolded.

A distinction is drawn between proteins which are soluble (i.e., dissolved) in a solution devoid of significant amounts of ionic detergents (e.g., SDS) or denaturants (e.g., urea, guanidine hydrochloride) and proteins which exist as a suspension of insoluble protein molecules dispersed within the solution. A soluble protein will not be removed from a solution containing the protein by centrifugation using conditions sufficient to remove bacteria present in a liquid medium (i.e., centrifugation at 5,000×g for 4–5 minutes). For example, to test whether two proteins, protein A and protein B, are soluble in solution, the two proteins are placed into a solution selected from the group consisting of PBS-NaCl (PBS containing 0.5M NaCl), PBS-NaCl containing 0.2% Tween 20, PBS, PBS containing 0.2% Tween 20, PBS-C (PBS containing 2 mM CaCl₂), PBS-C containing either 0.1 or 0.5% Tween 20, PBS-C containing either 0.1 or 0.5% NP-40, PBS-C containing either 0.1 or 0.5% Triton X-100, PBS-C containing 0.1% sodium deoxycholate. The mixture containing proteins A and B is then centrifuged at 5000×g for 5 minutes. The supernatant and pellet formed by centrifugation are then assayed for the presence of protein A and B. If protein A is found in the supernatant and not in the pellet [except for minor amounts (i.e., less than 10%) as a result of trapping], protein is said to be soluble in the solution tested. If the majority of protein B is found in the pellet (i.e., greater than 90%), then protein B is said to exist as a suspension in the solution tested.

As used herein, the term "therapeutic amount" refers to that amount of antitoxin required to neutralize the pathologic effects of one or more clostridial toxins in a subject.

The term "pyrogen" as used herein refers to a fever-producing substance. Pyrogens may be endogenous to the host (e.g., prostaglandins) or may be exogenous compounds (e.g., bacterial endo- and exotoxins, nonbacterial compounds such as antigens and certain steroid compounds, etc.). The presence of pyrogen in a pharmaceutical solution may be detected using the U.S. Pharmacopeia (USP) rabbit fever test (United States Pharmacopeia, Vol. XXII (1990) United States Pharmacopeial Convention, Rockville, Md., p. 151).

The term "endotoxin" as used herein refers to the high molecular weight complexes associated with the outer membrane of gram-negative bacteria. Unpurified endotoxin contains lipids, proteins and carbohydrates. Highly purified

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Entry information

Entry name **Q9R540_CLOBO**
 Primary accession number **Q9R540**
 Secondary accession numbers None
 Entered in TrEMBL in Release 13, May 2000
 Sequence was last modified in Release 13, May 2000
 Annotations were last modified in Release 25, October 2003

Name and origin of the protein

Protein name **Neurotoxin heavy chain 18 kDa fragment [Fragment]**
 Synonyms None
 Gene name None
 From **Clostridium botulinum [TaxID: 1491]**
 Taxonomy **Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae; Clostridium.**

References

[1] PROTEIN SEQUENCE.

MEDLINE=94000342;PubMed=8397793 [NCBI, ExPASy, EBI, Israel, Japan]
 Gimenez J.A., DasGupta B.R.;
 "Botulinum type A neurotoxin digested with pepsin yields 132, 97, 72, 45, 42, and 18 kD fragments.";
 J. Protein Chem. 12:351-363(1993).

Comments

None

Cross-references

HSSP **P10845; 3BTA. [HSSP ENTRY / PDB]**
 ProtoMap **Q9R540.**
 PRESAGE **Q9R540.**
 ModBase **Q9R540.**
 SMR **Q9R540; B7A959576A615E18.**
 SWISS-2DPAGE **Get region on 2D PAGE.**

UniRef [View cluster of proteins with at least 50% / 90% identity.](#)

Keywords

None

Features

None

Sequence information

Length: **72 AA** [This is the length of the partial sequence] Molecular weight: **8165 Da** [This is the MW of the partial sequence] CRC64: **B7A959576A615E18** [This is a checksum on the sequence]

10 20 30 40 50 60
 IYLNSSLYRG TKFIIKKYAS GNKDNIVRNN DRVYINVVVK NKEYRLATNA SQAGVEKILS

70
 ALEIPDVGNL YQ

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